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Characterization of the *Shigella* Serotype D (*S. sonnei*) O Polysaccharide and the Enterobacterial R1 Lipopolysaccharide Core by Use of Mouse Monoclonal Antibodies

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In the course of developing a live vaccine, we generated three murine monoclonal antibodies (MAb) specific for *Shigella sonnei*. The specificities of these MAb were determined by enzyme-linked immunosorbent assay and immunoblot analyses with whole cells or purified lipopolysaccharides (LPSs) as antigens. Two of them are specific for the *Shigella* serotype D O-polysaccharide determinant, whereas one specifically binds to the core hexose region of R1-type LPSs. With these MAb, it was possible to analyze clinical isolates and a hybrid *Salmonella typhi* strain for their expression of the corresponding LPS moieties. In addition to their use in the screening of candidate vaccine strains, the new MAb provide a powerful tool for epidemiological and phylogenetic studies of natural enterobacterial populations.

Shigellosis (bacillary dysentery) is endemic throughout the world. Especially in developing countries, this disease remains a major public health problem (2, 10), no satisfactory vaccine being available yet. It is, however, now widely accepted that efficient vaccine formulations should involve orally administrated live attenuated bacteria expressing the desired O serotype and able to invade the colonic mucosa to stimulate local and/or cellular immune responses (13, 25). Accordingly, efforts have been focused on the development of carrier strains for the expression of various *Shigella* O serotypes or on the engineering of *Shigella* strains with defined and stable attenuating mutations (13, 25, 33).

Lipopolysaccharide (LPS) molecules consist of three structural regions: a lipid A region, which anchors the molecule in the bacterial outer membrane; an intermediate core region; and a distal, serotype-specific O-polysaccharide chain (O chain or O antigen) (26, 31). Whereas the chemical composition and structure of O chains are highly variable, the core moiety is more conserved. Seven types of LPS core structures, namely, Ra, R1 to R4, K-12, and B, have been found in members of the family *Enterobacteriaceae* (15, 26).

Only one O-polysaccharide serotype, serotype D, is known for the species *Shigella sonnei* (25). Virulent cells (phase I) generate smooth colonies and express so-called form I LPSs; the latter comprise an R1-type core (11) associated with an O polysaccharide consisting of repeating disaccharide units (18). The genetic determinants for the *S. sonnei* O polysaccharide are encoded, together with other essential virulence determinants, on a large (180- to 200-kb) plasmid present in phase I cells (20, 34). Because of a high segregational instability of the virulence plasmid, smooth colonies of phase I cells spontaneously give rise to avirulent rough (phase II) variants expressing LPS molecules that lack the O polysaccharide (form II LPSs) (11).

Our goal is the generation of an efficient live attenuated anti-*Shigella* vaccine stably expressing the O antigen of *S. sonnei*. To this end, we have generated monoclonal antibodies (MAb) via immunization of mice with *S. sonnei* whole cells. In the present report, we describe the specificity of three MAb and show that they can be used to monitor the expression of *S. sonnei* O chain and core moieties in naturally occurring and genetically engineered bacteria. With these tools, it is now possible to screen for attenuated carrier strains expressing the corresponding epitopes and to analyze the stability of candidate vaccines.

We report for the first time MAb with a high specificity for *S. sonnei* LPS determinants, and these MAb may be very useful in epidemiological studies and as diagnostic tools. Of special interest is Sh9R, which will allow the determination of the frequency of LPS with an R1-type core–lipid A region among members of the family *Enterobacteriaceae*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in the characterization of LPS structures with MAb are listed in Table 1. Clinical *Escherichia coli* 204 (O1), 171 (O2), 133 (O6), 11 (O15), 60 (O25), and 3 (O75) were provided by A. Brauner, Karolinska Hospital, Stockholm, Sweden. *E. coli* 47 (O10), EC10 (O7), 253 (O12), and 205 (O18) were from the Walter Reed Army Institute of Research, Washington, D.C. The source of *Pseudomonas aeruginosa* strains (see Table 3) has been published already (23). Strains were grown in brain heart infusion medium (Difco Laboratories, Detroit, Mich.).

Production of MAb. Female BALB/c mice were immunized five times intraperitoneally with 5×10^8 to 10^9 heat-killed (1 h at 60°C) cells of *S. sonnei* phase I 53GI suspended in 500 μl of phosphate-buffered saline. Spleen immune cells were fused with the nonproducing myeloma cell line X63-Ag8.653 (17) as previously described (24). Hybridoma culture supernatants were assayed for the production of anti-LPS antibodies by an enzyme-linked immunosorbent assay (ELISA) (see below) with purified LPS. Supernatants were screened in parallel for antibody binding to LPSs from *S. sonnei* 53GI and 53GII. Positive hybridomas were cloned by limiting dilution. MAb classes and subclasses were deter-

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TABLE 1. Bacterial strains

Strain	O type	LPS structure	Expected core chemotype	Origin and comments
Shigella spp.				
S. sonnei 53GI	D	Form I	R1	Phase I (20)
S. sonnei 53GII		Form II	R1	Phase II variant of 53GI (this study)
S. sonnei 482-79(pWR105)	D	Form I	R1	P. Sansonetti; WR6050 (34)
S. sonnei PCM1985		Rough	R1	E. Romanowska
S. boydii 1	C1	Smooth	Unknown	Type 1; ATCC 9207
S. boydii 3	C3	Smooth	R1	Type 3; ATCC 8702
S. dysenteriae 1	A1	Smooth	R4	Type 1; ATCC 9361
S. flexneri 4b	B4b	Smooth	R3	Type 4b; ATCC 12024
S. flexneri 6	B6	Smooth	R1	Type 6; ATCC 12025
S. flexneri S323	B6	Smooth	R1	Type 6; E. Romanowska (30)
S. flexneri R6 551		Rough	R1	Deep rough mutant of *S. flexneri* type 6; E. Romanowska (16)
S. flexneri R6 288		Rough	R1	Same origin as R6 551
P. shigelloides				
M51	Like *S. sonnei*	Smooth	R1?	Bader; ATCC 14029
C27	Like *S. sonnei*	Smooth	R1?	Ferguson; ATCC 14030
E. coli				
HB101		Rough	K-12	3
HF4704		Rough	R1	A. A. Lindberg (15)
F2156		Rough	B	G. Schmidt (29)
O8:K8(L):H4	O8	Smooth	R1 or R2?	ATCC 23504
O9:K9(B):H12	O9	Smooth	R1	ATCC 23505
O14:K7(L):NM	O14	Smooth	R4	ATCC 19110
O100:H509a	O100	Smooth	R2	NCTC 9100
S. typhi				
TY21a	D1(9, 12)	Semirough[a]	Ra	*galE* (12)
TY37(pWR105)	D1(9, 12)	Semirough[a]	Ra	Rifampin-resistant derivative of TY21a bearing plasmid from 482-79(pWR105) (this study)
S. typhimurium TV119		Rough	Ra	K. E. Sanderson

[a] Semirough phenotype linked to the *galE* genetic background.

mined by an ELISA with a mouse typing kit (Bio-Rad Laboratories, Richmond, Calif.).

Purification of LPSs. LPSs were isolated by the hot phenol-water method of Westphal et al. (41) and further purified by RNase, DNase, and pronase treatments and by repeated ultracentrifugation rounds as previously reported (8). Purified LPSs of rough variants of *E. coli* (R4-type core) and *Salmonella typhimurium* (Rc- and Rd1-type cores) were purchased from Biocarb Chemicals, Lund, Sweden; according to supplier specifications, the preparations contained less than 3% protein and 5% nucleic acids.

LPS minipreparations. For fast-analysis purposes, LPSs were extracted from 1.5 ml of late-exponential- or stationary-phase liquid cultures by a rapid small-scale method (14).

SDS-PAGE and immunoblotting. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) analysis of LPS preparations was performed essentially as described by Laemmli (21) with either 15% or 7.5 to 20% acrylamide gels. LPS molecules were then silver stained by the procedure of Hitchcock and Brown (14).

For immunoblot analysis, LPSs separated on gels were electroblotted onto nitrocellulose filters (40). Blocking of the filters, incubation with antibodies, and enzymatic detection of the immunoreactive material were done essentially as described previously (24).

For immunodot analysis of whole bacterial cells, the latter were washed and resuspended in the same volume of phosphate-buffered saline, and 5 μl was spotted onto nitrocellulose filters. The spots were allowed to dry at room temperature and then processed as for LPS immunoblots, starting from the blocking step, except that incubations with the first and second antibodies were each done for 30 min at room temperature.

ELISA. Binding assays were performed as described previously (4), with the exception that coating with LPSs was done for 2 h at 37°C and was followed by overnight incubation at 4°C. Peroxidase-conjugated affinity-purified goat anti-mouse immunoglobulin M (IgM) antibodies (Tago, Burlingame, Calif.) were used as developing antibodies.

Competitive binding assays were performed as described previously (4). The capacity of commercially available (Sigma Chemical Co., St. Louis, Mo.) disaccharides to inhibit the binding of MAb Sh9R was tested in plates coated with 0.5 μg of *S. sonnei* 53GII LPSs per well. Tested disaccharides were 3-O-β-D-galactopyranosyl-D-arabinose, 3-O-α-D-glucopyranosyl-D-fructose [D-(+)-turanose], 4-O-α-D-glucopyranosyl-D-glucose [D-(+)-maltose], 1-O-α-D-glucopyranosyl-D-fructose [D-(+)-sucrose], 6-O-α-D-glucopyranosyl-D-fructose (palatinose), 4-O-β-D-galactopyranosyl-D-glucose [D-(+)-lactose], 4-O-β-D-galactopyranosyl-D-fructose (lactulose), 6-O-β-D-glucopyranosyl-D-glucose (β-gentiobiose; 95% β-anomer), 1-O-α-D-glucopyranosyl-α-D-glucose [D-(+)-tre-

TABLE 2. Binding of anti-*S. sonnei* LPS MAb

MAb	Isotype[a]	Titer for *S. sonnei* variant of phase[b]:		Immunoblot pattern for *S. sonnei* variant of phase[c]:		
				I		II (form II LPSs)
		I	II	Form I LPSs	Form II LPSs	
Sh5S	IgM	>128	<2	+	−	−
Sh11S	IgM	>128	<2	+	−	−
Sh9R	IgM	16	64	−	+	+

[a] Determined by an ELISA as described in Materials and Methods.
[b] Determined by an ELISA in wells coated with LPSs purified from phase I or phase II variants of *S. sonnei* 53G. Titers are expressed as the reciprocal of the dilution resulting in an optical density of 0.4.
[c] Binding (+) or lack of binding (−) to slowly migrating O-antigen-bearing form I or fast-migrating form II LPS components in immunoblots of LPS preparations run on SDS-polyacrylamide gels.

halose], and 1-O-β-D-glucopyranosyl-D-glucose [D-(+)-cellobiose].

RESULTS

Hybridoma cell lines secreting murine IgM MAb directed against *S. sonnei* LPS molecules were generated from mice immunized with heat-killed whole cells of *S. sonnei*. Three stable cell lines were selected for study. The binding of these MAb to LPSs isolated from smooth (phase-I) and rough (phase-II) variants of *S. sonnei* 53G is shown in Table 2. MAb Sh5S and Sh11S exhibited high ELISA titers for the high-molecular-weight form I LPSs consisting of core-lipid A and O-polysaccharide moieties but did not react with the low-molecular-weight form II LPSs lacking the O-polysaccharide moiety. In contrast, MAb Sh9R bound to both kinds of molecules, with a preference for the form II LPSs. Consistently, immunoblot analysis revealed that Sh5S and Sh11S bound to O-polysaccharide-bearing LPSs, whereas Sh9R specifically reacted with the core-lipid A moiety.

The specificity of MAb Sh5S and Sh9R was further examined by immunoblot analysis of LPSs isolated from a panel of bacterial strains of known O-antigen specificity and LPS core structures (Fig. 1).

O-polysaccharide-specific MAb Sh5S (Fig. 1B) recognized LPS molecules from different phase I strains of *S. sonnei* (lanes b and d) as well as from strains M51 and C27 of *Plesiomonas shigelloides* (lanes k and l), reported to belong to the same O serotype as *S. sonnei* (1, 32). For all these strains, MAb Sh5S bound only to slowly running form I LPSs bearing O polysaccharide. Furthermore, O-antigen determinants expressed by the *S. typhi* hybrid strain TY37 (lane t) were recognized. We constructed this strain via conjugation and mobilization of Tn*5*-tagged *S. sonnei* virulence plasmid pWR105 from strain 482-79(pWR105) into *S. typhi* live oral vaccine strain TY21a (12) by use of mobilizing replicon R386 (34).

When MAb Sh9R was used to probe the same LPS preparations (Fig. 1C), only strains reported to have LPS molecules with an R1-type core structure (Table 1) were recognized, namely, *S. sonnei* (lanes b to d), *S. boydii* type 3 (lane g), *S. flexneri* type 6 (lanes i and j), a control *E. coli* R1 strain (HF4704; lane m), and an *E. coli* O9 isolate (lane o). To our knowledge, the LPS core structure of *S. boydii* type 1 has not been described yet; data obtained with reference strain ATCC 9207 (lane f) were compatible with the presence of R1-type LPSs in this strain. Two possible

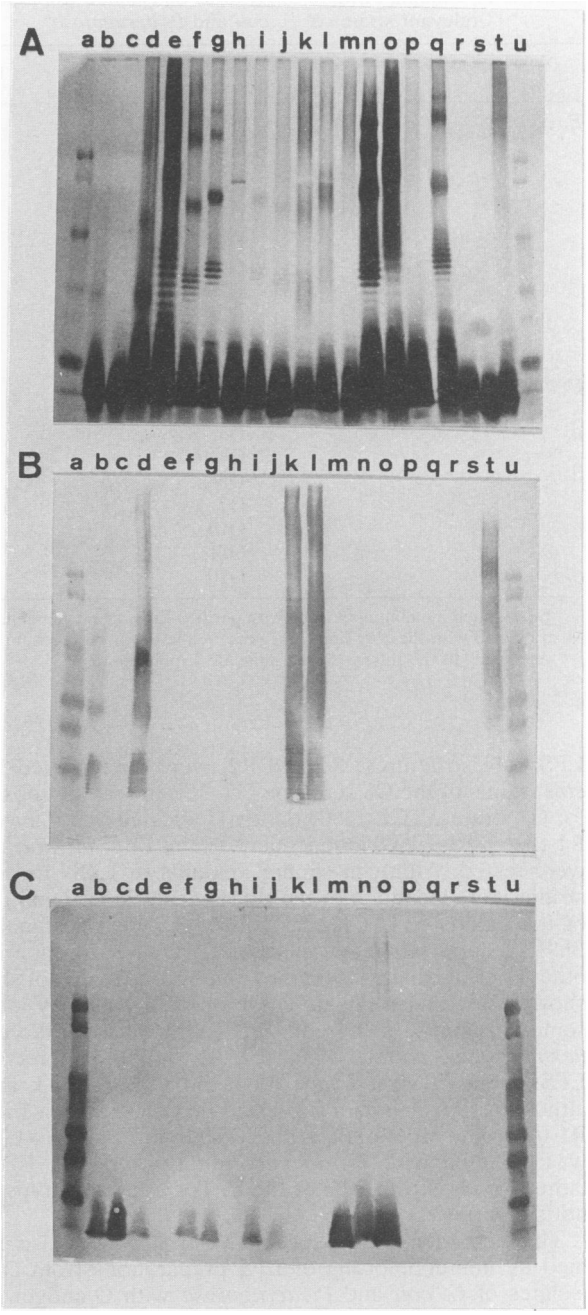

FIG. 1. Reactivity of MAb Sh5S and Sh9R for LPSs isolated from various enterobacterial species. (A) Silver staining of LPS minipreparations run on a 7.5 to 20% gradient SDS-polyacrylamide gel. (B and C) Immunoblot analysis with Sh5S (B) and Sh9R (C). Lanes: a and u, prestained low-molecular-weight protein standards (Bio-Rad); b, *S. sonnei* 53GI; c, *S. sonnei* 53GII; d, *S. sonnei* 482-79 (pWR105); e, *S. dysenteriae* type 1 (ATCC 9361); f, *S. boydii* type 1 (ATCC 9207); g, *S. boydii* type 3 (ATCC 8702); h, *S. flexneri* type 4b (ATCC 12024); i, *S. flexneri* type 6 (S323); j, *S. flexneri* type 6 (ATCC 12025); k, *P. shigelloides* M51 (ATCC 14029); l, *P. shigelloides* C27 (ATCC 14030); m, *E. coli* HF4704; n, *E. coli* O8 (ATCC 23504); o, *E. coli* O9 (ATCC 23505); p, *E. coli* O14 (ATCC 19110); q, *E. coli* O100 (NCTC 9100); r, *E. coli* HB101; s, *S. typhi* TY21a; t, *S. typhi* TY37(pWR105::R386).

TABLE 3. Binding of MAb Sh9R to LPSs isolated from clinically relevant strains of *E. coli* and *P. aeruginosa*[a]

Bacterial species	Serotype	Binding
E. coli	O1	+
	O2	+
	O6	+
	O7	+
	O10	−
	O12	−
	O15	−
	O18	+
	O25	+
	O75	+
P. aeruginosa	O1[b]	−
	O2	−
	O3	−
	O4	−
	O6	−
	O7	−
	O10	−
	O11	−
	O16	−

[a] Determined by immunoblotting with purified LPSs as the antigen. +, the MAb reacted with the core-lipid A region; −, there was no reaction.
[b] According to the International Antigenic Typing System.

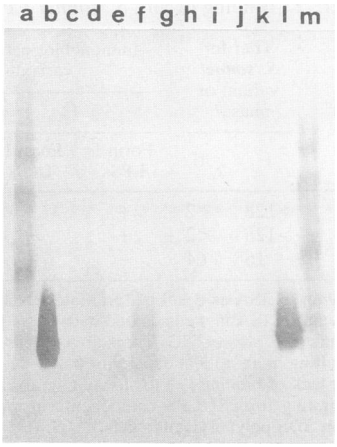

FIG. 2. Immunoblot analysis with MAb Sh9R of LPS preparations from rough mutant strains with structural differences in the hexose region of the core. Identical amounts (5 μg) of each preparation were loaded on a 15% polyacrylamide gel, except in lanes d and f (25 μg). Lanes: a and m, molecular weight standards (see Fig. 1); b, *S. sonnei* 53GII; c and d, *S. flexneri* R6 551; e and f, *S. flexneri* R6 288; g, *E. coli* R4 (Biocarb); h, *E. coli* K-12 (HB101); i, *S. typhimurium* Ra (TV119); j, *S. typhimurium* Rc (Biocarb); k, *S. typhimurium* Rd1 (Biocarb); l, *E. coli* R1 (HF4704).

LPS core structures, R1 and R2, were documented for *E. coli* strains of the O8 serotype (35, 36); the pattern observed for O8 strain ATCC 23504 (lane n) indicated that it may bear R1-type LPSs. Two independent strains of *S. flexneri* type 6 were tested. Although similar amounts of LPSs from both strains were loaded onto the gel (Fig. 1A, lanes i and j), one of the strains (ATCC 14025) showed a weaker signal with Sh9R (lane j), whereas the second strain (S323; lane i) and other clinical isolates from the same source (data not shown) showed a stronger signal. These observations may indicate some variability in the core-lipid A regions of different *S. flexneri* type 6 isolates. Finally, MAb Sh9R did not recognize LPSs from *P. shigelloides* M51 and C27 (lanes k and l), although published data suggested that these strains have an R1-type core (see Table 1 and Discussion), and no binding was detected with *Vibrio cholerae* serotype O1 LPSs of either the classical Inaba or the El Tor Ogawa subtype (data not shown).

The reactivity of core-specific MAb Sh9R was also examined by immunoblotting of LPS preparations from clinical isolates of *E. coli* and *P. aeruginosa* with O-antigen serotypes among the most frequently encountered in nosocomial cases of bacterial sepsis (6, 7, 28) (Table 3). Among the *E. coli* strains, 7 of 10 (with O1, O2, O6, O7, O18, O25, and O75 serotypes) showed a strong reaction, indicating that despite their wide range of O-antigen serotypes, they bear LPSs with an R1-type core structure. In contrast, LPS preparations from nine clinical isolates of *P. aeruginosa* failed to react with MAb Sh9R.

According to published serological studies (11), the terminal hexose sugars of *S. sonnei* phase II LPSs may correspond to essential elements of the R1-type immunodeterminant. In an attempt to map more precisely the epitope for MAb Sh9R, we compared LPS preparations from several rough mutant strains with known structural differences within the core hexose region in an immunoblotting experiment (Fig. 2); relevant LPS structures are depicted in Fig. 3. Strong binding to LPSs isolated from *S. sonnei* phase II strain 53GII and *E. coli* HF4704 (Fig. 2, lanes b and l) was observed. Both strains are characterized by the R1-type LPS core hexose region. MAb Sh9R was also positive for the R1-type LPS core of *S. flexneri* type 6 (see above and Fig. 1). The structure of the *S. flexneri* type 6 core hexose region is identical to that of the *S. sonnei* core hexose region, with a possible difference in the anomeric configuration of the innermost D-glucose residue. Interestingly, the LPSs of *S. flexneri* type 6 rough mutant strain R6 551 (Fig. 2, lanes c and d), which lack the lateral D-glucose residue in the hexose region, and the LPSs of the *E. coli* R4 type (lane g), in which a lateral D-galactose residue replaces D-glucose in an otherwise identical hexose sugar backbone, were not recognized. These results point to the latter residue as being part of the epitope recognized by Sh9R. *S. flexneri* type 6 rough mutant strain R6 288 (Fig. 2, lanes e and f) consistently provided a signal intermediate between those of strain R6 551 and *S. sonnei* 53GII or *E. coli* HF4704. Thus, R6 288 LPSs, characterized by a terminal D-glucose residue in a β(1-3) linkage to the core heptose region, may have a lower-affinity epitope for MAb Sh9R. Consistent with the latter observation, a pattern similar to that for R6 288 was observed for *E. coli* B strain F2156 (data not shown), in which the LPS core hexose region is limited to a diglucose residue (26). Other LPS preparations showed no signal or weak signals.

To evaluate the epitope size, we determined the capacity of different disaccharides to competitively inhibit the binding of MAb Sh9R to *S. sonnei* 53GII LPSs. Whether they contained D-glucose or not (see Materials and Methods), high concentrations of disaccharides were required to obtain a measurable effect. Indeed, for the disaccharides 3-O-β-D-galactopyranosyl-D-arabinose, D-(+)-turanose, D-(+)-maltose, D-(+)-sucrose, palatinose, D-(+)-lactose, lactulose, β-gentiobiose, D-(+)-trehalose, and D-(+)-cellobiose, concentrations of between 1.5 mg/ml (3-O-β-D-galactopyranosyl-D-arabinose) and 29 mg/ml [D-(+)-trehalose]) were required to achieve 50% inhibition of Sh9R binding to *S.*

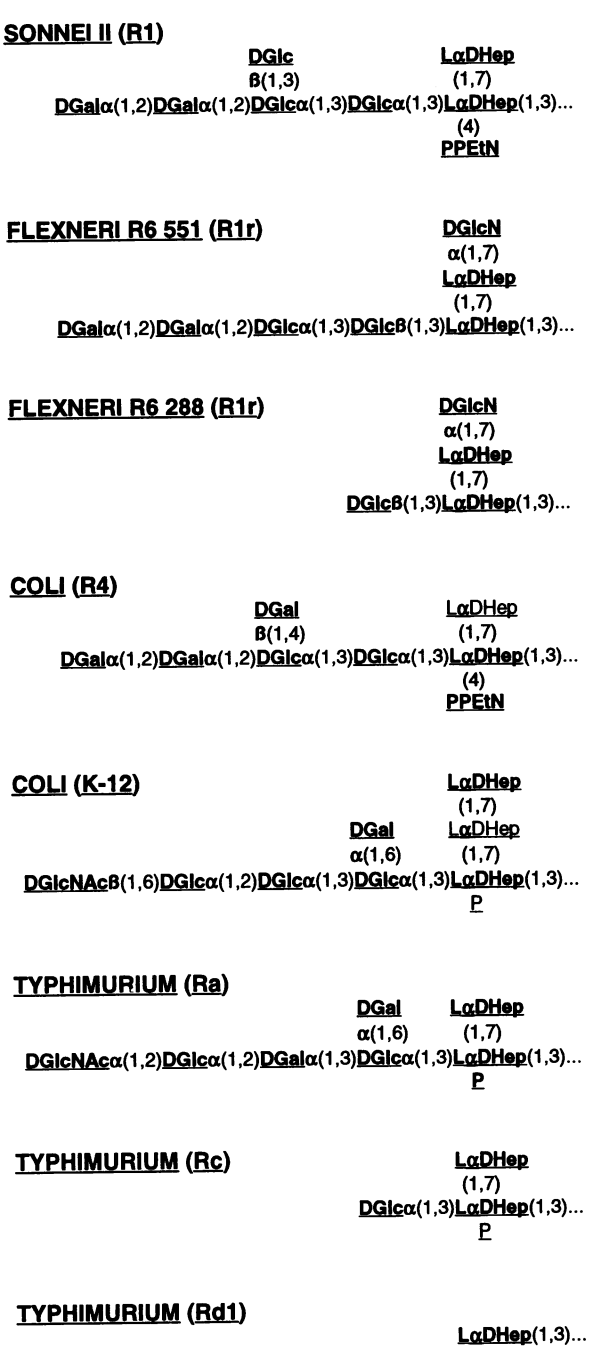

FIG. 3. Structures of the LPS hexose regions of relevant strains used in the characterization of R1-type core-specific MAb Sh9R (Fig. 2). For clarity, the outermost heptose residue of the inner core region is also depicted with its various lateral substitutions. The structures shown represent a compilation from references 15, 16, and 26. The LPS core type is indicated in parentheses following the species designation. DGlc, D-glucose; DGal, D-galactose; LαDHep, L-glycero-D-mannoheptose; DGlcN, D-glucosamine; DGlcNAc, *N*-acetyl-D-glucosamine; PPEtN, pyrophosphorylethanolamine; P, phosphate. Sugar residues and other substituents are in boldface type. Non-boldface type indicates some uncertainty either in the published structures, as for the lateral LαDHep residue of the *E. coli* K-12 and R4 cores, or about the presence of one specific substituent in the available LPS preparation, as for the phosphate group in the *S. typhimurium* Rc core.

sonnei 53GII LPSs. In contrast, 23 μg of the homologous 53GII LPS per ml resulted in 50% inhibition.

DISCUSSION

MAb have proven to be very powerful tools in molecular biology. In many cases, only such reagents allow an efficient screening for the expression of cloned genetic determinants. In the present report, we show that our newly generated MAb are very efficient in the detection of *Shigella* serotype D and R1-type LPS determinants expressed in both naturally occurring and recombinant strains.

S. typhi TY37 is a hybrid derivative of live vaccine strain TY21a (12) bearing the entire *S. sonnei* virulence plasmid. TY37 is essentially equivalent to previously reported strain 5076-1C (9), with the further advantage that the virulence plasmid can be more stably maintained via selection for kanamycin resistance. Chemical analyses of the *S. sonnei* products expressed by strain 5076-1C suggested that the O antigen was present on the cell surface as a polymer of O-disaccharide repeating units without covalent linkage to the core-lipid A region (37). Using MAb Sh5S, we provided evidence that TY37, like 5076-1C, expresses the *S. sonnei* O-chain determinants in a form unbound to the *S. typhi* Ra-type LPS core. Indeed, the recognized material failed to be detected by silver staining and did not show the typical LPS ladder observed on immunoblots for *S. sonnei* phase I and *P. shigelloides* strains. In addition, the bulk of this material was lost during LPS purification (data not shown).

Tested isolates with reported R1-type LPS core structures were specifically recognized by MAb Sh9R. This consistency enabled us to unambiguously identify unknown LPS structures. However, Sh9R did not bind to LPSs isolated from *P. shigelloides* M51 and C27, although published serological and chemical analyses suggested that their overall structures are similar to that of *S. sonnei* (1, 32). The observed binding defect may stem from the fact that the *P. shigelloides* LPS core bears at least one (strain M51) or two (strain C27) sugar substitutions (32). Finally, the nonrecognition of *V. cholerae* O1 LPSs was expected from published studies showing that its chemical composition presents some striking differences from the compositions commonly found in gram-negative bacterial LPSs (19, 39).

Because of their high levels of specificity, the new MAb will be most useful as diagnostic tools. Sh9R may especially be important as a defined anti-R1-type core reagent. Indeed, in comparison with the impressive amount of data accumulated on the epidemiology of *E. coli* O serotypes (28), little is known about the distribution of the six reported core chemotypes (26, 28). Our study revealed that LPSs from 7 of 10 *E. coli* strains with O serotypes most frequently found in nosocomial infections were bound by Sh9R. These results indicate that the R1-type core is predominant among clinically relevant *E. coli* strains. Finally, Sh9R may also be a useful addition to the methodology used in phylogenetic studies of natural *E. coli* populations (38).

The pattern of reactivity of Sh9R with various rough LPS preparations differing in the hexose region of the core suggests that the lateral D-glucose (Fig. 3) is an essential part of the epitope. Interestingly, it was reported that the latter residue, together with the distal D-galactose-α(1-2)-D-galactose disaccharide (Fig. 3), may correspond to the structural determinant of the R1 serotype, whereas the core heptose region does not seem to be involved in serological specificity (11). The fact that the same D-glucose residue is the target for the covalent binding of the *S. sonnei* O antigen (11) may

account for the nondetection of *S. sonnei* LPSs substituted with the O-antigen moiety (form I LPSs) in immunoblots with Sh9R. Similarly, epitope masking by the O-polysaccharide chain was reported for specific MAb directed against the core hexose domain of *Salmonella* LPSs (27).

Although the present study does not allow an unambiguous determination of the Sh9R epitope, both immunoblot analyses and competitive binding studies with disaccharides suggest that the epitope spans more than a mono- or disaccharide. This suggestion is in agreement with the observation that human anti-*Klebsiella pneumoniae* MAb specifically recognize clusters of capsular polysaccharide serotypes with no obvious homology in their primary structures (22). Similarly, complex MAb epitopes involving adjacent and nonadjacent sugar residues were recently identified at the molecular level within the *Salmonella* LPS core (27). In contrast, an anti-*P. aeruginosa* MAb with specificity for the core glycolipid LPS region was reported to have an affinity for a mixture of totally hydrolyzed LPS monosaccharides (5), and the binding of an outer core-specific MAb was inhibited by L-rhamnose monosaccharide (42).

Finally, it is noteworthy that MAb Sh5S and Sh9R were instrumental in the cloning of the genetic determinants for the *S. sonnei* O serotype and the R1-type LPS core from generated cosmid gene banks (unpublished data), an important step toward the development of a live vaccine against *S. sonnei*.

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